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Silício no manejo de doenças do arroz e elaboração e validação de uma escala diagramática para mancha ocular

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Pelotas, 2020

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A meu Filho
Jerónimo Rivera B

À minha avó
Leonora Zuleta (in memorian)

Dedico

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Resumo

Hernández, Juan Felipe Rivera. **Silício no manejo de doenças do arroz e elaboração e validação de uma escala diagramática para mancha ocular.** 2019. 48f. Tese (Doutorado) – Programa de Pós-Graduação em Fitossanidade. Universidade Federal de Pelotas. Pelotas, RS, Brasil.

O arroz (*Oryza sativa*) é uma cultura de importância econômica para muitos países por ser considerado como alimento estratégico para segurança alimentar tornando-se primordial para a humanidade. Dentre os diversos fatores que limitam sua produtividade estão as doenças que quando não manejadas podem acarretar danos na produção até 100%. Diante disso, os objetivos desta pesquisa foram: i) avaliar o efeito da adubação silicatada (Si) nos componentes de resistência [período de incubação (PI), eficiência relativa de infecção (ERI), taxa de expansão de lesão (*r*), comprimento final de lesão (CFL), período latente (PL), área abaixo da curva do progresso da doença (AUDPC) e severidade final (SF)] à mancha ocular (*Drechslera gigantea*) nas cultivares BRS Querência (EMBRAPA), BRS Atalanta (EMBRAPA), suscetíveis, e Puitá INTA CL (BASF S/A); e avaliar o efeito da adubação silicatada, em condições de campo, na severidade da mancha parda (*Bipolaris Oryzae)*, brusone (*Magnaporthe oryzae*) e escaldadura (*Monographella albescens*)*,* produtividade e qualidade industrial dos grãos de arroz; ii) desenvolver e validar uma escala diagramática para quantificação da severidade da mancha ocular em folhas do arroz utilizando o software Quant. Nos resultados em casa de vegetação, os fatores Si e cultivar foram significativos, mas não as suas interações. O fornecimento de Si às plantas reduziu o PI, PL, ERI, CFL, SF e AUDPC, respectivamente em 8,4, 23,4, 33,11, 28,12, 62,23, 61,86%, comparado as plantas sem Si. Em campo, o fornecimento de Si reduziu a AUDPC da mancha parda e da escaldadura em 25,7 e 16,4%, respectivamente. Não ocorreu diference significativa no rendimento entre cultivares ou tratamentos com Si. Na qualidade industrial de grãos, observou-se que não houve diferença significativa entre as cultivares e nem efeito Si. A escala diagramática proposta contém sete níveis de severidade (0,3; 1,0; 3,0; 5,0; 10; 15; e 21%), verificando-se que as estimativas na quantificação da severidade da mancha ocular foram mais precisas e acuradas. Em conclusão, os resultados apresentados neste trabalho mostram que a aplicação de silicato de cálcio no solo resultou no acúmulo Si nas folhas de arroz o que incrementou a resistência à *Drechslera gigantea* na cultura do arroz, entretanto em condições de campo o efeito da fertilização silicatada foi menos expressivo.

Palavras chave: *Drechslera gigantea*, Silicato de cálcio, Escala diagramática, Acurácia e precisão, Segurança alimentar, *Oryza sativa*.

Abstract

Hernández, Juan Felipe Rivera. **Silicon in rice disease management and elaboration and validation of a diagrammatic scale for ocular staining.** 2019. 48f. Tese (Doutorado) – Programa de Pós-Graduação em Fitossanidade. Universidade Federal de Pelotas. Pelotas, RS, Brasil.

Rice (*Oryza sativa*) is a crop of economic importance for many countries because it is considered as a strategic food for food security, making it paramount for humankind. Among the several factors that limit rice productivity are diseases that when unmanaged can lead to damages up to 100%. The objective of this research was: i) to evaluate the effect of silicate fertilization (Si) on the resistance components [incubation period (IP), relative efficiency of infection (REI), lesion expansion rate (*r*), lesion length (LL), latent period (LP), area under disease progress curve (AUDPC) and final severity (FS)] to the eye leaf spot (*Drechslera gigantea*) in cultivars BRS Querência (EMBRAPA), BRS Atalanta (EMBRAPA)) and Puitá INTA CL (BASF S / A); and evaluate the effect of silicon fertilization in field on brown spot (*Bipolaris oryzae*), blast (*Magnaporthe oryzae*) and scald (*Monographella albescens*), grains yield and its industrial quality; ii) to develop and validate a diagrammatic scale for quantification of eye leaf spot severity using the Quant software. In greenhouse results, the factors Si and cultivar were significant, but not their interactions. The supply of Si to the plants reduced IP, LP, REI, LL, FS and AUDPC, respectively at 8.4, 23.4, 33.11, 28.12, 62.23, 61.86%, compared to plants without Si. In field, the AUDPC of the brown spot and scald was reduced by 25.7 and 16.4%, respectively, in Si-treated plants. No significant difference was observed in grains yield or its quality due cultivars or Si treatment. The proposed diagrammatic scale contains seven levels of severity (0.3, 1.0, 3.0, 5.0, 10, 15, and 21%) and it increased the precision and accurate for estimation of the eye leaf spot severity. In conclusion, the results presented in this work show that the application of calcium silicate in the soil resulted in Si accumulation in the rice leaves, which increased the resistance to *Drechslera gigantea*. However, in field the effect of silicon fertilization was less expressive.

Keywords: *Drechslera gigantea*, Calcium silicate, Diagrammatic scale, Accuracy, Food safety, *Oryza sativa*.

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Sumário

1 Introdução Geral

O arroz (*Oryza sativa* L) é considerado uma das culturas de maior importância a nível mundial, devido a ser fundamental nos programas de segurança alimentar para mitigar a fome no mundo. A China é o maior produtor (208 milhões de toneladas), seguida pela Índia (165 milhões de toneladas) e Indonésia (74 milhões de toneladas) (FAOSTAT, 2018). Na América Latina, o Brasil é o maior produtor, com mais de 12 milhões de toneladas em uma área de 2 milhões de hectares, ocupando o terceiro lugar em área cultivada entre as culturas anuais (CONAB, 2017). Apesar de estes reportes, a produção deste cereal é limitada pela ocorrência de doenças que afetam o seu rendimento (SOSBAI, 2016).

Doenças como brusone (*Pyricularia grisea*), mancha parda (*Bipolaris oryzae*), escaldadura da folha (*Gerlachia oryzae*) e queima das bainhas (*Rhizoctonia solani*), continuam sendo limitantes, ocasionando danos de até 100% na produtividade, quando não são manejadas apropriadamente (NUNES, 2013). Além disso, doenças emergentes têm sido reportadas recentemente, como: mal-do-pé (*Gaeumannomyces graminis* var. *graminis*), carvão da Folha (*Etyloma oryzae*) e mancha ocular (*Drechslera gigantea*) (NUNES, 2013), sendo esta última o foco principal deste trabalho.

O fungo *Drechslera gigantea* foi identificado pela primeira vez em 1909, como *Helmintosporium giganteum* em grama bermuda (*Cynodon dactylon* L.), através do resultado de um levantamento de doenças de 293 plantas silvestres em San Antônio Texas, EUA (HEALD e WOLF, 1911). As lesões causadas nas folhas se caracterizam pela presença de manchas de cor amarela ou pálida, longitudinalmente alargadas e com uma borda marrom. Os conidióforos descritos são de cor marrom escuro, com uma base ligeiramente bulbosa e os esporos longos, cilíndricos, com os extremos ligeiramente cónicos com 5 septos, de cor pardo claro (AHN, 1980).

Os primeiros relatos da mancha ocular no arroz, foram realizados por AHN (1980), quem reportou a ocorrência de *Drechslera gigantea* na cultivar CICA 7 no Panamá, CICA 6 na Colômbia, e em Naylamp no Perú. O autor explica que sob condições favoráveis, como períodos prolongados de umidade nas folhas, as lesões coalescem ocupando a maior área do limbo foliar. Quando foram avaliados os danos na produção, não houve um efeito significativo no rendimento, porém poderia tornar-se limitante se as condições ambientais favoráveis como umidade prolongada nas folhas, aparecerem durante o ciclo da cultura (AHN, 1980).

No Brasil, a mancha foi identificada pela primeira vez no Estado de Rio Grande do Sul, na safra de 2006/07, e sua incidência foi observada nas safras na região Sul e Depressão Central do RS, bem como no Estado do Mato Grosso, principalmente na cultivar BRS Querência, no final do estádio de grãos pastosos, sem causar danos na produtividade (NUNES, 2008).

No manejo de doenças de maneira geral, a quantificação é de grande importância no sentido de avaliar diferentes medidas de controle, resistência varietal e teste da eficácia de produtos fitossanitários, porém, sua utilização é de difícil execução, por ser um procedimento trabalhoso e relativamente dispendioso (RODRIGUES et al., 2002; GOMES et al., 2004; VALE et al., 2004). A forma mais adequada de quantificar doenças é por meio da severidade, a qual representa o percentual de tecido foliar doente em relação à área foliar (AMORIM, 2018). Apesar da subjetividade a que está sujeita a avaliação visual da severidade, esta deve fornecer dados acurados, precisos e reproduzíveis (VALE et al., 2004). A utilização de escalas diagramáticas pode reduzir a subjetividade das estimativas de severidade entre os avaliadores, melhorando a acurácia e precisão da avaliação (LEITE e AMORIM, 2002; RODRIGUES et al., 2002; MARTINS et al., 2004). No entanto, as escalas têm que ser reproduzíveis e devem apresentar níveis suficientes para caracterização do avanço no progresso da doença (GODOY et al., 2006).

Na elaboração de escalas diagramáticas devem ser considerados os seguintes fatores: o limite superior da escala deve corresponder à quantidade máxima de doença observada em campo e sua representação na escala devem ter alta precisão; as subdivisões da escala devem respeitar as limitações da acuidade visual humana de acordo com a lei de Weber-Fechner; e lembrar que a visão humana enxerga tecido doente para níveis de severidade abaixo de 50% e tecido sadio para níveis de severidade superiores a 50% (HORSFALL e COWLING, 1978; GOMES et al., 2004). O processo de validação das escalas é extremadamente importante, pois é a garantia de que é realmente eficaz em gerar estimativas precisas, acuradas e reproduzíveis. (DUARTE et al., 2014). Atualmente, são recomendadas novas ferramentas para a elaboração e validação de escalas diagramáticas. Dentre elas podemos citar o uso de valores lineares de severidade nos diagramas, o maior número de diagramas e novas análises para a validação, como o teste de correlação e concordância de Lin (NITA et al., 2003, DUARTE et al., 2014; BOCK et al., 2016) Atualmente existem disponíveis no mercado programas computacionais que auxiliam na quantificação de doenças, como o QUANT, desenvolvido pela Universidade Federal de Viçosa, que por meio da avaliação de imagens, permite determinar a área foliar lesionada de forma precisa e acurada (VALE et al., 2003).

Entre as diferentes ferramentas disponíveis para o manejo de doenças em plantas, a utilização de fungicidas é uma das principais formas de controle, porém, um recurso disponível é a utilização dos agentes potencializadores ou indutores de defesas na planta, sendo que, dentre eles, o silício (Si) vem se destacando para o controle de várias doenças de plantas (DATNOFF, 2005).

O Si é absorvido pelas raízes na forma neutra, como ácido monosilíco (H_4SiO_4), na forma passiva ou ativa, através de transportadores de membrana específicos para este fim (MA et al., 2006). O transporte do ácido monosilícico é feito via xilema, a favor de um fluxo de transpiração, sendo movido, em maior parte, até a parte aérea da planta, onde é depositado em forma de sílica amorfa (SiO_2), acumulando-se e aumentando a espessura, em diferentes tecidos vegetais, principalmente da parede de células foliares (OLIVEIRA et al., 2010).

No controle de doenças, o Si atua nos mecanismos de defesa que envolvem desde barreira física à penetração de patógenos pela deposição do Si e polimerização na cutícula e na parede celular (HUANG et al., 2011; GUERRIERO et al., 2016) bem como pela ativação de respostas bioquímicas de defesa da planta por meio da ativação de enzimas de defesa (POLANCO et al., 2014; DORNELES et al., 2017), produção de compostos fenólicos (TATAGIBA et al., 2014), fitoalexinas (HAYNES, 2017) e lignina (POLANCO et al., 2012), e a nível molecular, pela regulação da expressão de genes envolvidos na defesa e resposta ao estresse (GHAREEB et al., 2011; WANG et al., 2017).

No caso do arroz, são vários os relatos da utilização da fertilização com Si no aumento da resistência da planta a diversas doenças como a brusone, a mancha-parda, a podridão-do-colmo (*Magnaporthe salvinii*), a queima das bainhas e a descoloração os grãos (DATNOFF, 1991; DATNOFF et al., 1997). Sendo assim, o objetivo deste trabalho foi desenvolver uma escala diagramática para a quantificação da severidade da mancha ocular, assim como avaliar a eficácia do silício nos componentes de resistência da doença em casa de vegetação, e verificar o seu efeito nas principais doenças do arroz irrigado em campo no Município do Capão de Leão RS.

2 Artigo 1

Calcium silicate affects diseases but not yield and quality of rice

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Calcium silicate affects diseases but not yield and quality of rice

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Abstract

Rice (Oryza sativa L.) is a crop of economic importance for many countries because it is considered as a strategic food for food security, making it paramount for humankind. Among the several factors that limit its productivity are diseases that when unmanaged can lead to production damages. The objectives of this study were to evaluate:1) the effect of silicon (Si) fertilization for rice plants in cultivated areas of Rio Grande do Sul (RS) – Brazil to diseases control, under flooding and; 2) the effect of Si on the resistance of rice plants to eyespot. The cultivars BRS Querência, BRS Atalanta and Puitá INTA CL were studied using calcium silicate (CS) as Si source. The field was evaluated the occurrence of blast, brown spot, leaf scald, leaf Si accumulation, grains yield and its quality and, in a greenhouse were evaluated the components of eyespot resistance and leaf Si accumulation. The application of CS in the soil reduced brown spot and leaf scald severity but the rice response to CS fertilization was undetectable in grains yield and its quality. The application of CS in the soil under greenhouse conditions resulted in Si accumulation in the rice leaves which increased the resistance to eyespot.

Key words: *Drechslera gigantea* - *Oryza sativa* – Epidemiology – Food Safety – Calcium Silicate

Rice (*Oryza sativa* L.) is one of the mostly produced and consumed cereals worldwide being estimated an increase, up to 60%, in its demand by 2050 (Faostat 2018). Brazil is among the ten main rice producers, and achieved a production of 10.49 mi tonnes in 2018/19, being the State of Rio Grande do Sul responsible for 60% of Brazilian rice production (Conab, 2019).

The rice plant is susceptible to pathogens that compromise the development and production by lowering the number and weight of grains, besides the reduction in grains nutritional quality by lowering the carbohydrates and/or proteins content (Malavolta et al. 2002; Santos et al. 2002; Prabhu et al. 2003; Shabana et al. 2008). In Brazil, the main rice diseases are blast (*Magnaporthe oryzae* Hebert), brown spot (*Cochliobolus miyabeanus Ito & Kuribayashi*), leaf scald (*Monographella albescens* Thuemen) and sheath blight (*Thanatephorus cucumeris* Frank) (Sosbai 2016). Resistant cultivars, healthy seeds and fungicide application are the main control alternatives used for rice diseases management (Groth 2006; Wang et al. 2007; Sosbai 2016).

The silicon (Si) is reported in many studies as an element that reduces rice diseases and increase grains yield and quality (Dallagnol et al. 2015; Rodrigues et al. 2015); however, in some regions the Si fertilization has been shown to provide any impact on rice resistance to disease and grain yield (Marchezan et al. 2004).

Whereas the Rio Grande do Sul is the main rice producer in Brazil, and the Si is not part of the disease management strategies employed for rice producers, in this study we evaluate the calcium silicate (Si source) fertilization effects on rice diseases intensity and in their progress, as well as the effects of calcium silicate on rice yield and quality. Furthermore, as eyespot (*Pyrenophora gigantea*) was reported to occur in some rice cultivars growth in the South of

Brazil (Nunes 2013), the effects of Si on the resistance components of rice plants to eyespot were also investigated.

Two independent experiments were conducted, being the first at field and the second at greenhouse level. Field experiment was conducted in order to evaluated the response of flooded rice plants to soil fertilization with calcium silicate to control of blast, brown spot and leaf scald. The experimental area used is located in Capão do Leão, RS (31°48'28.5"S 52°28'51.9"W). Greenhouse experiment was conducted in order to evaluate the Si effects on the rice resistance against eyespot. Seeds from the rice cultivars BRS Querência (EMBRAPA), BRS Atalanta (EMBRAPA), and Puitá INTA CL (BASF S/A) were used in for both field and greenhouse experiments. Grains from the three used genotypes are categorized as long-slender and high amylose. Adjustments in soil fertility and weeds control were performed according to the regional crop management recommendations (Sosbai 2016). The concentration of available Si (extracted with 0.01 M $CaCl_2$) in the soils used in the field and greenhouse experiments were 14.0 and 6.0 mg dm^{-3}, respectively.

In the field experiment, the experimental design was a two-way factorial in a block randomized design (three cultivars and two silicon treatments - not supplied (Si-) or supplied (Si+) treatments) with four replications. Each replication consisted of 10 m^2 (2 × 5m) being considered as useful area 4.8 m^2 (1.2 × 4m). Experimental unit was separated each other by 100 cm of distance and surrounded by ridge (40 cm tall and 60 cm wide). The experiment was conducted twice.

The source of Si was calcium silicate (Agrosilício®), which was composed of 25.0% calcium, 6.0% magnesium and 10.5% Si, at a rate of 4.9 tonnes ha^{-1}. To standardize the amount of calcium and magnesium supplied to the plants in the calcium silicate treatment, soil of the control treatment was amended with limestone, composed of 26.5% calcium and 15.0% magnesium, at a rate of 4.1 tonnes ha^{-1}. Calcium silicate or limestone were incorporated into the soil using a rotary hoe 30 days before sowing. In the second year (experiment repetition), the products were applied again to keep the soil pH at 6.5.

Data of average rainfall, air humidity, and maximum and minimum daily temperatures were obtained from the Meteorological Station of the Brazilian Agricultural Research Corporation (EMBRAPA) Terras Baixas, Capão do Leão, RS (31°48'12"S, 52°24'40"W - altitude of 13.24m).

Diseases assessment was initiated after seedling emergence and repeated weekly until harvesting. The diseases (blast, brown spot and leaf scald) incidence were recorded at the reproductive phase. For diseases assessment, the severity (percentage of total leaf area affected by each diseases) was evaluated on all flag leaves of 12 marked plants and systematically positioned inside each plot to represent as best as possible the area of the replication. Leaf blast was assessed using a 10-level scale (0, 0.5, 1.0, 2.0, 4.0, 8.0, 16.0, 32.0, 64.0 and 82.0% of affected leaf area) according to Notteghem (1981); brown spot was assessed using a 7-level scale (0.1, 0.5, 1.0, 3.0, 6.5, 11.8 and 28.8 % of affected leaf area) proposed by Schwanck and Del Ponte (2014); and leaf scald was assessed using a 6-level scale (0, 1, 1-5, 6-25, 26-50 and 50 -100%) as described by IRRI (2002). Data from disease severity was plotted to obtain the disease progress curves and the area under disease progress curve (AUDPC) was calculate according to Shaner and Finney (1977).

The rice grains were harvested from one square meter of each plot when the moisture content was approximately 22%. Grains were then subjected to cleaning and drying processes until 13% of moisture content was achieved. Afterwards, rice yield was estimated and expressed as kg ha^{-1}. The brown rice content and the head rice yield were determined according to the Brazilian Rice Regulation (Brasil 2009). For this, rice samples (100 g) were subjected to dehusking and milling using a Zaccaria Rice Machine. Subsequently, the polished kernels were placed in the trieur component of the same Zaccaria Rice Machine where the broken kernels were separated from the whole kernels. Broken grains were those with a length lower than ¾ parts of the minimum length of 6.0 mm accepted for long grains, ie less than 4,49 mm (Brasil 2009). The brown rice content and the head rice yield were expressed as percentage. The polished rice samples were subjected to chalky and translucid grains identification and separation. For each sample, totally translucid grains were weighted and their percentage calculated.

In the last assessment of disease severity, the flag leaves were sampled and used for the to determine the Si concentration as described in Pazdiora et al. (2018).

In the greenhouse experiment, six treatments were arranged in a fully randomized two-way factorial: three cultivars (BRS Querência, BRS Atalanta and Puitá INTA CL) and application of Si (with or without application) with six replications. Each experimental unit consisted of a plastic pot containing 4 kg of soil. Rice seeds were sowed in plastic pots and after seedling emergence, they were thinned to four plants per pot. Plants were kept inside a greenhouse during the experiment, with daily mean temperature ranging between 25 and 35 °C. The greenhouse experiment was also conducted twice.

Calcium silicate (Agrosilício®) and extra-fine limestone (control) were mixed with the soil at a rate of 11.86 and 10.98 g kg^{-1} of soil, respectively. Calcium carbonate and magnesium carbonate were used to adjust the concentration of calcium and magnesium, respectively. After complete soil homogenization in each treatment, water was added to reach 80% of field capacity, followed by 30 days of incubation in plastic bags.

An isolate of *Drechslera gigantea* (PLS04), provided by the Laboratory of Plant Pathogenic Fungi and Seeds Pathology (Eliseu Maciel Agronomy School / Federal University of Pelotas), was used in the experiment. The fungal growth media used was V8 which composition is described in Dorneles et al. (2017). After fungal growth in the V8 media for seven days, the conidia were collected in sterile water containing 0.01% Tween 20 and the concentration was adjusted to 6×10^4 conidia mL^{-1}. This suspension was sprayed onto leaves of rice plants at R_1 phenological stage (according to Counce et al. 2000) after 53 days after sowing, as a fine mist using a hand-held sprayer, without runoff. Immediately after inoculation, the plants were kept for 48 hours inside a humid chamber with relative humidity above 90%, temperature of 25 ± 2 ºC and photoperiod of 12 hours.

The following resistance components were evaluated on leaves of each plant: incubation period (IP), relative infection efficiency (RIE), rate of lesion expansion (*r*), final lesion size (FLS), latent period (LP), area under disease progress curve (AUDPC) and final severity (SEV). The IP (hours), RIE, *r*, FLS were evaluated as described previoulsly (Dorneles et al. 2017; Pazdiora et al. 2018). The LP (hours) was assessed by examining the leaves every six hours after IP using a magnifier with 20 × amplification and was defined as the interval between inoculation and visualization of fungal conidia. Disease severity, defined as the percentage of total leaf area affected by the disease (necrotic and chlorotic tissue) was estimated each 48 hours after IP using a standard area diagram set (Rivera, 2019). The severity data over time were used to calculate AUDPC according to Shaner and Finney (1977). Immediately after the last disease assessment, at 23 days after inoculation, leaves were digitalized using a scanner (HP / C4280), and actual disease severity (final severity) was measured using the digital image analysis software Quant (Vale et al. 2003). These leaves were used for Si quantification according to procedures previously described.

To evidence the normality of the data, the Shapiro Wilk test was applied. Whenever the interaction was not significant, the model was reduced to test only the main effects. Whenever a main factor was significant ($P < 0.05$), treatment means were compared based on Student test (*t*-test) ($P = 0.05$) or Tukey test ($p \leq 0.05$). All analysis were conducted using SAS software (SAS Institute 1989, Cary, NC).

In field experiment, the interaction of factors was not significant ($P \geq 0.05$) for all variables. The leaf concentration of Si was not influenced ($P \geq 0.05$) by cultivar or soil corrective (Fig. 1A). For diseases, in 2016/17 crop season, the occurrence of brown spot, leaf scald and blast were recorded. These diseases occurred only at reproductive phase and their progress was affected by cultivar and soil corrective, especially for those that occurred earlier: brown spot (Fig.1C) and leaf scald (Fig. 1D), but not for blast (Fig. 1B) that occurred at the end of crop cycle and with low severity. In the case of brown spot, especially in plants of the cultivars BRS Querência and BRS Atalanta, disease progress in +Si plants was slowed, resulting in final severity 11.5- and 12.8%-lower than -Si plants (Fig. 1C). Furthermore, calcium silicate application reduced the AUDPC by 25.7 and 16.4%, respectively for brown spot (Fig. 1C) and leaf scald (Fig. 1D). Among the cultivars, the higher AUDPC for brown spot (Fig. 1C) and for leaf scald (Fig. 1D) was determined for BRS Atalanta.

In the crop season 2017/2018 no diseases were recorded during the crop cycle, probably due to less favorable weather conditions (Supplemental material S1), especially linked to irregular rainfall during the reproductive phase.

Grains yield (Fig. 1E) and the measured rice quality attributes (Fig. 1F-H) were not significantly influenced by cultivars or soil corrective.

For eyespot, the interaction of factors was not significant ($P > 0.05$) for all variables; however, one or two main effects significantly affected the treatment means, depending on the variable (Table 1). For the cultivar Puitá INTA CL, symptoms of the disease were not visualized throughout the experiment, being considered resistant to eyespot. Cultivars (BRS Atalanta and BRS Querencia) were different only for ERI being this variable 40% lower for BRS Querencia (Table 1). Silicon (+Si) increased the IP and LP in 7 and 23%, and reduced the ERI, FLS and SEV in 33, 28 and 62%, compared to plants -Si (Table 1). Together, silicon effect on resistance components resulted in 62% of reduction in the AUDPC. Leaf concentration of Si was 51% higher in plants +Si compared to -Si plants (Table 1).

In field studies, calcium silicate fertilization did not result in significant increase in the Si concentration in the soil or the plant tissues. This result is due to the high concentration of available Si in the soil where the experiment was conducted and the adopted cultivation system (flooding), in this case probably due to alteration in the redox potential of the soil leading to a high release of Si, thus supplying the minimum plant demands. Previous studies demonstrated that Si concentration in the soil solution is affected by the redox potential, pH and interaction with other soil minerals (Liang et al. 2015; Tubaña and Heckman 2015).

Calcium silicate fertilization, although not increased Si concentration in plants tissue, reduced the AUDPC of the brown spot and leaf scald during the 2016/17 crop season. However, the low severity of the diseases and the occurrence in the later stages of crop development did neither affected grain yield nor the rice quality attributes of brown rice content, head rice yield and translucid rice content. In the 2017/18 crop season, diseases were not recorded, probably due to unfavorable environmental conditions for pathogens infection. In this case, the beneficial effect of calcium silicate fertilization was not observed neither on grain yield nor its quality. According to Cooke and Leishman (2016), Si has little effect on plant metabolism under normal conditions, but its beneficial effect is observed under stress (biotic and abiotic) conditions. Thus, the flooded rice, reducing water deficiency to plants, added to the low biotic stresses and high Si release from soil particles, which increased the element availability in soil solution, mitigated the effects of calcium silicate fertilization on rice plants.

Greenhouse experiments, regarding the resistance of the cultivars to eyespot, revealed the cultivar Puitá INTA CL as completely resistant, due no symptoms of the diseases were visualized. Considering that *D. gigantea* is a necrotrophic pathogen (Green et al. 2004), the resistance observed in the cultivar Puitá INTA CL should be further investigated, at both molecular and biochemical levels, for a better understanding of the infection inhibition mechanism. On the other hand, BRS Atalanta and BRS Querência cultivars were susceptible to the pathogen, although the BRS Querência showed higher resistance to the establishment of the fungus as indicate by the lower RIE.

For the soil used in the greenhouse experiment, calcium silicate fertilization increased Si concentration in leaf. This higher leaf Si concentration, in susceptible cultivars, reduced the eyespot intensity, as evidenced by the increase in IP and LP, and reduction in the variables RIE, FLS, SEV and AUDPC. The IP and LP tend to vary in parallel, ie the increase in the IP should reflect the increase in the LP and consequently reducing the secondary cycles of infections and disease progress (Parlevliet 1979). Moreover, Si accumulation also reduced the RIE, indicating a smaller number of lesions per unit of area as well as reduced the *r* resulting in lower FLS. Taken together the effects of Si on the various monocyclic components of the eyespot resulted in a significant reduction in final disease severity and disease progress over time as evidenced by the AUDPC. For rice several studies showed similar effects of Si on other pathogens (Rodrigues et al. 2015; Debona et al. 2017). Thus, the availability of soluble Si in the soil solution in concentration necessary to supply the rice plant demand is an alternative to reduce the damage caused by the eyespot. Flooded fields may naturally provide the required amount of Si, however in rice upland fields the calcium silicate fertilization may be required to supply the Si demand of rice plants.

In conclusion, the results presented in this work show that the application of calcium silicate in the soil reduced brown spot and leaf scald severity but under flooded field, due to the soil characteristics and flooding-induced soil chemical alterations, the rice response to calcium silicate fertilization was undetectable in grains yield and rice quality attributes of brown rice content, head rice yield and translucid grains content. The application of calcium silicate in the soil under greenhouse conditions resulted in Si accumulation in the rice leaves which increased the resistance to eyespot.

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Figures

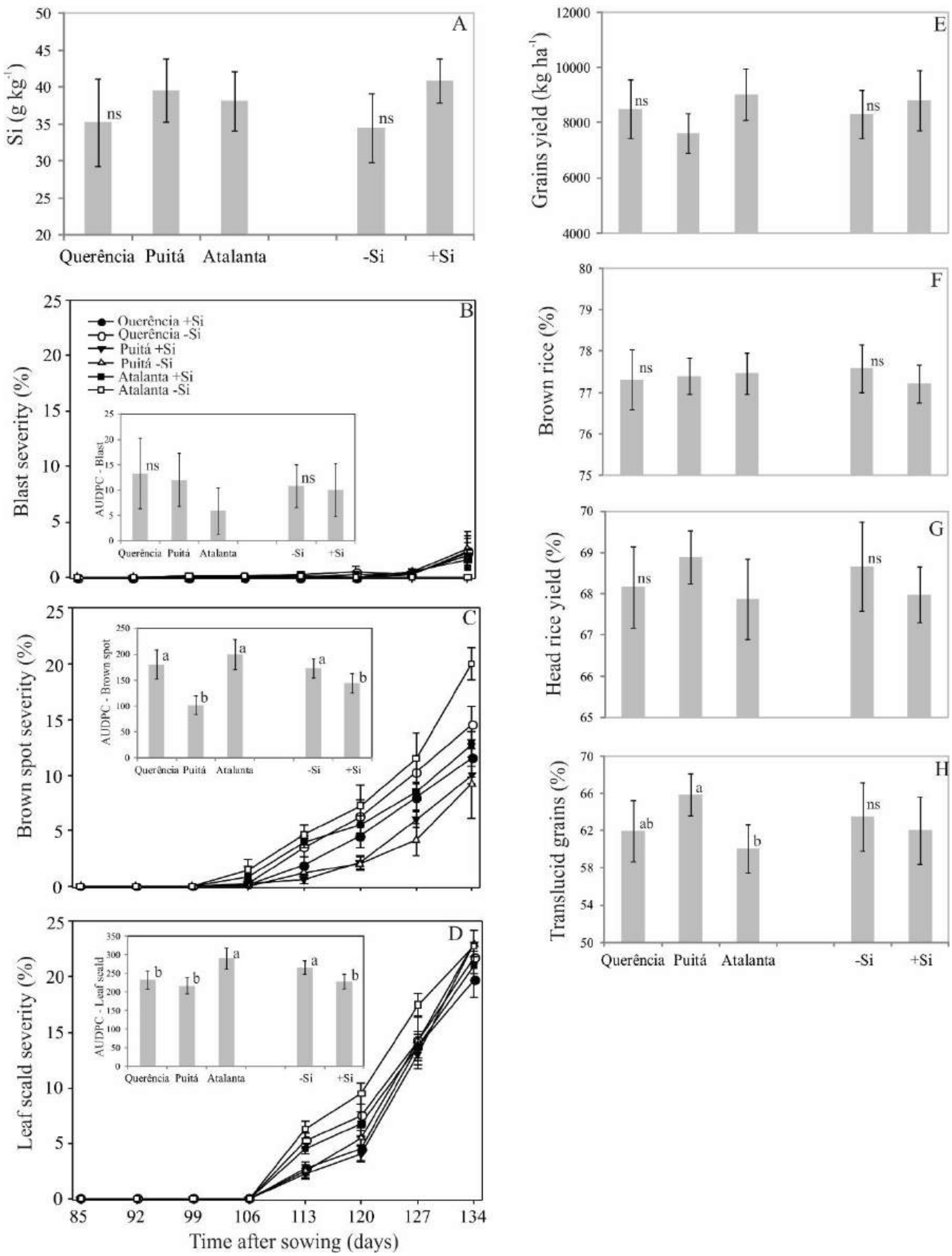


Figure 1. Leaf silicon (Si) concentration (A), progress curve and area under disease progress curve (AUDPC) of blast (B), brown spot (C) and leaf scald (D), grains yield (E), brown rice

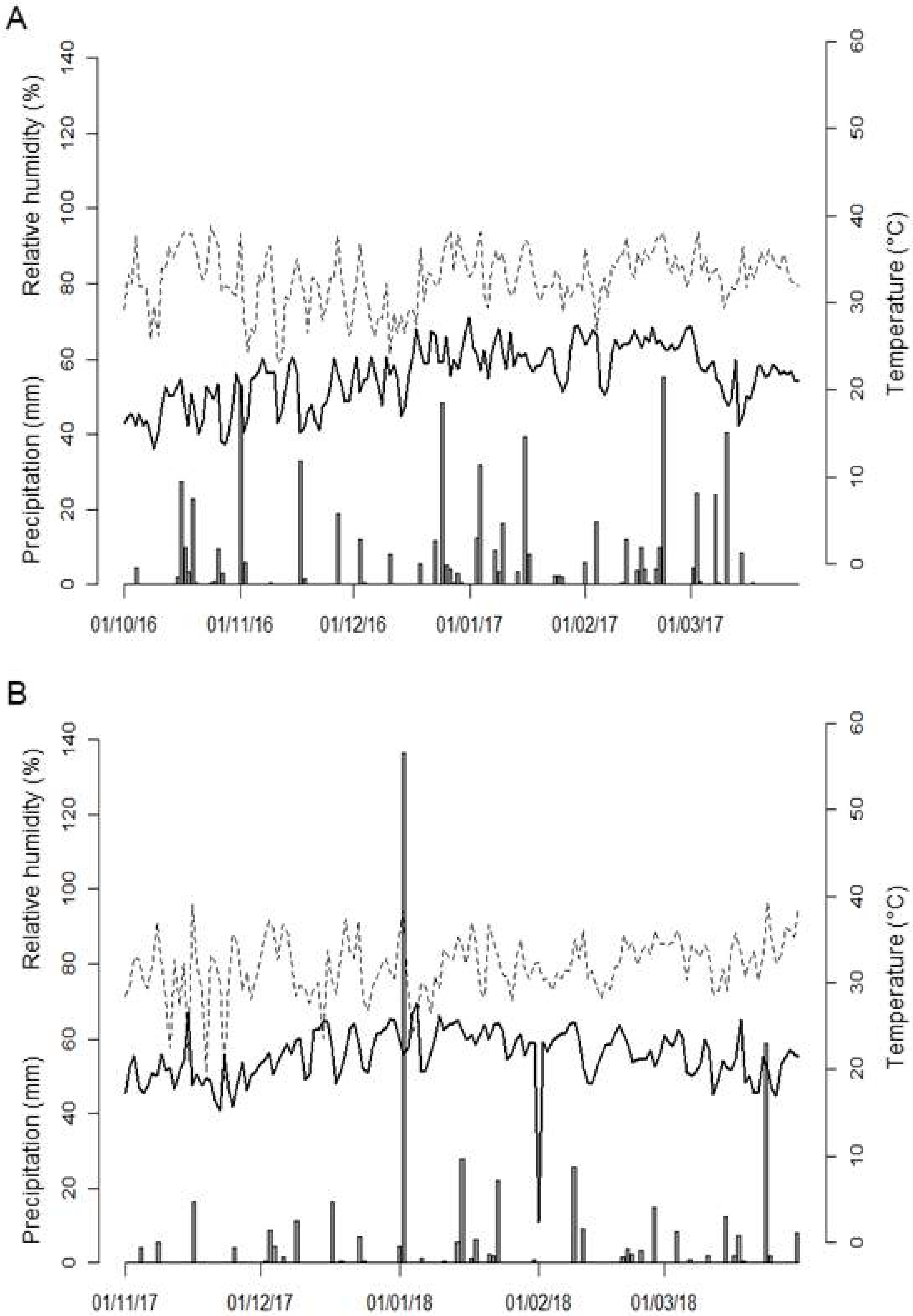
(F), head rice yield (G) and translucid grains (H) from rice plants of the cultivars BRS Querência, Puitá INTA CL and BRS Atalanta, grown in flooded soil containing calcium silicate (+Si) or extra-fine limestone (-Si). Data of the two crop seasons were combined in the figures A, E, F, G and H. Data in the figures B, C and D were from 2016/17 crop season.

Tables

Table 1 Incubation period (IP), latent period (LP), relative infection efficiency (RIE), lesion expansion rate (*r*), final lesion size (FLS) final severity (SEV), area under disease progress curve (AUDPC) and silicon (Si) concentration in leaves of BRS Atalanta e BRS Querência cultivated in soil supplied with limestone (-Si) or calcium silicate (+Si) and inoculated with *Drechslera gigantea*.

Cultivars	**IP (h)**	**LP (h)**	**RIE (%)**	***r***	**FLS (mm)**	**SEV (%)**	**AACPD**	**Si (g. kg^{-1})**
BRS Atalanta	23.08 ^{ns}	82.41 ^{ns}	29.05 *	0.0141 ^{ns}	3.07 ^{ns}	7.77 ^{ns}	231.96 ^{ns}	11.29 ^{ns}
BRS Querência	23.33	83.91	17.34	0.0116	2.31	7.73	216.65	10.78
Treatments								
- Si	22.41 *	72.16 *	27.79 *	0.0144 ^{ns}	3.13 *	11.25 *	324.77 *	8.74 *
+ Si	24.00	94.16	18.59	0.0112	2.25	4.25	123.84	13.24
CV%	5.02	4.63	35.09	36.18	24.78	12.20	13.77	13.98

*Significant difference by *t*-test (p = 0.05). ns= not significant. Data of the two experiment repetitions were combined.



Supplemental material S1. Precipitation (bars), relative humidity (dashed line) and average air temperature (black line) in the experimental area in the agricultural years 2016/17 (A) e 2017/18 (B).

3 Artigo 2

A standard area diagram set for severity assessment of eyespot on rice

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A standard area diagram set for severity assessment of eyespot on rice

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Abstract

This study aimed to develop and validate a standard area diagram set (SADs) to estimate the severity of eyespot of rice caused by *Drechslera gigantea*. For this purpose, a SADs with seven levels of severity (0.3; 1.0; 3.0; 5.0; 10; 15; and 21%) was established. The SADs was validated by 16 raters with no experience in evaluating plant diseases. Both accuracy and precision improved when they used the SADs. The statistical parameters for the 16 raters were: bias coefficient factor - C_b (no SADs = 0.404, with SADs = 0.994); correlation coefficient - r (no SADs = 0.884, with SADs = 0.953); and Lin's concordance correlation coefficient - ρc (no SADs = 0.356, with SADs = 0.947). In addition, estimates were more reliable: inter-rater coefficient of determination - R^2 (no SADs = 0.712, with SAD = 0.873); intra-class correlation coefficient - ρ (no SADs = 0.723, with SADs = 0.924). The SADs proposed here is a useful tool for improving visual assessments of eyespot severity of rice.

Keywords *Oryza sativa* · *Drechslera gigantea* · Disease assessment · Phytopathometry

Rice (*Oryza sativa* Linn.) is the principal food for more than 50% people and contributes about one-fifth to the total calories consumption of the world (Singh et al. 2012). In 2017, globally rice crop occupies 158 million hectares of the arable land. The global production and productivity of rice was about 745 million tonnes and 4.71 tonnes ha^{-1}, respectively (FAO 2019). Rice is grown over a widely divergent environments, such as irrigated uplands and lowlands, rainfed lowlands, and rainfed upland ecosystems (Choudhary and Suri, 2014; Kaur et al. 2015). In Brazil, rice production occurs as irrigated lowlands, mainly in the Rio Grande do Sul State, which responsible for 70% of national rice production (Conab 2019).

The challenges and constraints in rice production vary from environment to environment. Biotic stresses are one of the major problems that affect the rice yields to the greatest extent (Paul et al. 2014), regardless of the cropping ecosystems. The average yield losses in rice due biotic stress are estimated to vary between 40 and 60% which may reach up to 94 - 96 % without crop protection (Chauhan and Johnson 2011).

Rice eyespot caused by *Drechslera gigantea* (Heald et Wolf) S. Ito (Ito 1930) is a foliar disease, and it has been reported in several Latin American countries (Ahn 1980; Nunes 2008) and the United States (Kardin et al. 1982). In the South of Brazil, the disease was reported in the 2006/07 season, and since then the eyespot has been observed in all crop seasons, in some specific cultivars, mainly after the early dough stages of grain development (Nunes et al. 2013).

The pathogen infects the rice leaves causing oval lesions with a grayish center and reddish-brown margin, 0.5 - 2.5 × 0.2 - 1.0 mm (Fig. 1). Dematiaceous conidia 150 to 550 µm long × 15 to 18 µm wide with 3 to 12 septa, borne singly on unbranched conidiophores, are observed in these lesions (Nunes et al. 2013). Conidia showed bipolar germination with multiple germ tubes. Though conidia are produced on rice leaves, diseased weed, host of the fungus, growing on footpaths between rice fields also contribute producing numerous conidia of the pathogen (Sato et al. 1990). When the diseased weed is located adjacent to rice plants, more than 50 spots are often observed on a rice leaf. So far, damage in the yield is not clear, especially in Brazil where the disease has been observed occurring in the late stage of plant development. However, the effect of the disease may become significant if it occurs in the vegetative stage and/or the environmental conditions become favorable to the pathogen, such as prolonged leaf wetting, at the beginning of the crop season.

Among the main management strategies of rice diseases, genetic control is one of the most important alternatives at the present time, although chemical control is often employed in the management programs of fungal diseases in rice. In the case of an emerging disease will be necessary studies to identify resistant genotype, epidemiological requirements to develop the epidemy and evaluation of control measures. For this, it is of great importance to establish a method that allows measure the disease amount, more accurate, precise and with reliable estimative of disease severity in those studies.

The use of standard area diagram set (SADs) is a way of visually estimating of plant disease severity (Bock et al. 2010). Despite the growing relevance of eyespot of rice, there is no SADs published to assess the disease, which will be very useful for several purposes, including breeding for resistance, fungicide screening, and pathotype characterization.

The aim of this study was to develop and validate a SADs to estimate the severity of eyespot of rice, providing a useful tool for the evaluation of the disease.

One hundred fifty leaves of rice with symptoms of eyespot were sampled at the experimental area of Federal University of Pelotas, State of Rio Grande do Sul, Brazil, during the 2017/18 season. The symptomatic leaves were randomly collected from epidemical trials of cultivars, which allowed the manifestation of the disease in different levels of severity.

The symptomatic leaves were individually digitized at a resolution of 300 dpi, using a scanner (HP® ScanJet 2400; Hewlett-Packard, Alto, CA, USA). For each leaf, the proportion of diseased area was determined using the QUANT software (Vale et al. 2003). The SAD illustrations were chosen from these samples with adjustments made by the Paint program (Microsoft® Office 365), establishing a set of seven disease severity levels, in a linear distribution (Nutter Jr and Esker 2006; Bock et al. 2010). Lesions with a grayish center, reddish-brown margin and leaf necrosis were quantified as diseased area. Foliar chlorosis was not considered.

The SADs was validated by 16 raters with no previous experience in plant disease severity assessments. Fifty images of diseased leaves were displayed as a slide show in PowerPoint to the raters. Each image was displayed for one minute and the raters were then asked to write down the estimated percentage of diseased area on a form. For the second assessment, they evaluated the same 50 images again in a different sequence, but with the aid of the SADs developed in this study. The accuracy, precision and inter-rater reliability of the estimates with and without the SADs were calculated as previously described (Dolinski et al. 2017), using the R software (R Core Team 2017).

Seven illustrations, covering the minimum (0.3%) and the maximum (21%) of eyespot severity observed in the field, comprised the SADs (Fig. 2).

All statistical parameters (υ, u, C_b, r, and ρc) of Lin's concordance correlation (LCCC) were significantly improved when the raters used the SADs to estimate disease severity, demonstrating that both the accuracy and precision of the estimated values were improved. The statistical parameters values were: scale bias - υ (no SADs = 2.700, with SADs = 0.980) (confidence intervals = -2.000 - -1.441), location bias (u) (no SADs = 1.463, with SADs = -0.024) (confidence intervals = -1.718 - -1.260), bias coefficient factor - C_b (no SADs = 0.404, with SADs = 0.994) (confidence intervals = 0.533 - 0.647); correlation coefficient - r (no SADs = 0.884, with SADs = 0.953) (confidence intervals = 0.051 - 0.086); and Lin's concordance correlation coefficient - ρc (no SADs = 0.356, with SADs = 0.947) (confidence intervals = 0.541 - 0.641). As the CI did not embrace zero, the difference was significant ($\alpha = 0.05$). Based on estimated and actual severity, assessments made by the raters were closer to the actual values using the SADs (Fig. 3 a and b). The absolute error of the estimates reduced significantly when the raters used the SADs (Fig. 3 c and d).

Inter-rater reliability of assessments by 16 raters was significantly improved. Without the SADs, the intra-class correlation coefficient mean (ρ) was 0.723 (confidence intervals = 0.619-0.814), while using the SADs, this value was 0.924 (confidence intervals = 0.893-0.951). In turn, the mean of inter-rater coefficient of determination (R^2) of the pairwise comparisons were 0.712 (minimum = 0.458, maximum = 0.863) and 0.873 (minimum = 0.764, maximum = 0.946) without and with SADs, respectively. The 95% confidence interval (CI) of this mean was 0.145 to 0.177. As the CI did not embrace zero, the difference was significant ($\alpha = 0.05$).

The SADs developed in this study improved accuracy, precision, and reliability of the estimations of eyespot severity. The number of diagrams in this set is similar to that in the SADs developed for brown spot on rice (Schwanck et al. 2012), thereby facilitating assessment for the raters. Moreover, each diagram in this study showed the entire leaf, which prevented errors related to patterns of disease distribution on the leaves.

Despite the availability of software with high accuracy and precision in the quantification of diseases severities, such as Quant (Vale et al. 2003), APS Assess 2.0: Image Analysis Software for Plant Disease Quantification, and Leaf Doctor (Pethybridge and Nelson 2015), SADs are still a powerful tool to improve the accuracy and precision of experienced or inexperienced raters (Lage et al. 2015; Nuñez et al. 2017). In addition, SADs does not require specific conditions of image acquisition, nor people with specific training, and it is practical for assessments of large amounts of samples.

SADs for rice eyespot can assist plant pathologists, breeders and extensionists in quantifying the disease, in a way of more accurate and reliable estimates of disease severity in studies involving epidemiological analyses, evaluating disease management strategies and genotypes selection for resistance to the disease.

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Figures

Fig. 1 Rice leaves of the cultivar BRS Atalanta affected by eyespot during an epidemic year of the disease (A); Details and pattern of eyespot symptoms distribution on rice leaves (B and C).

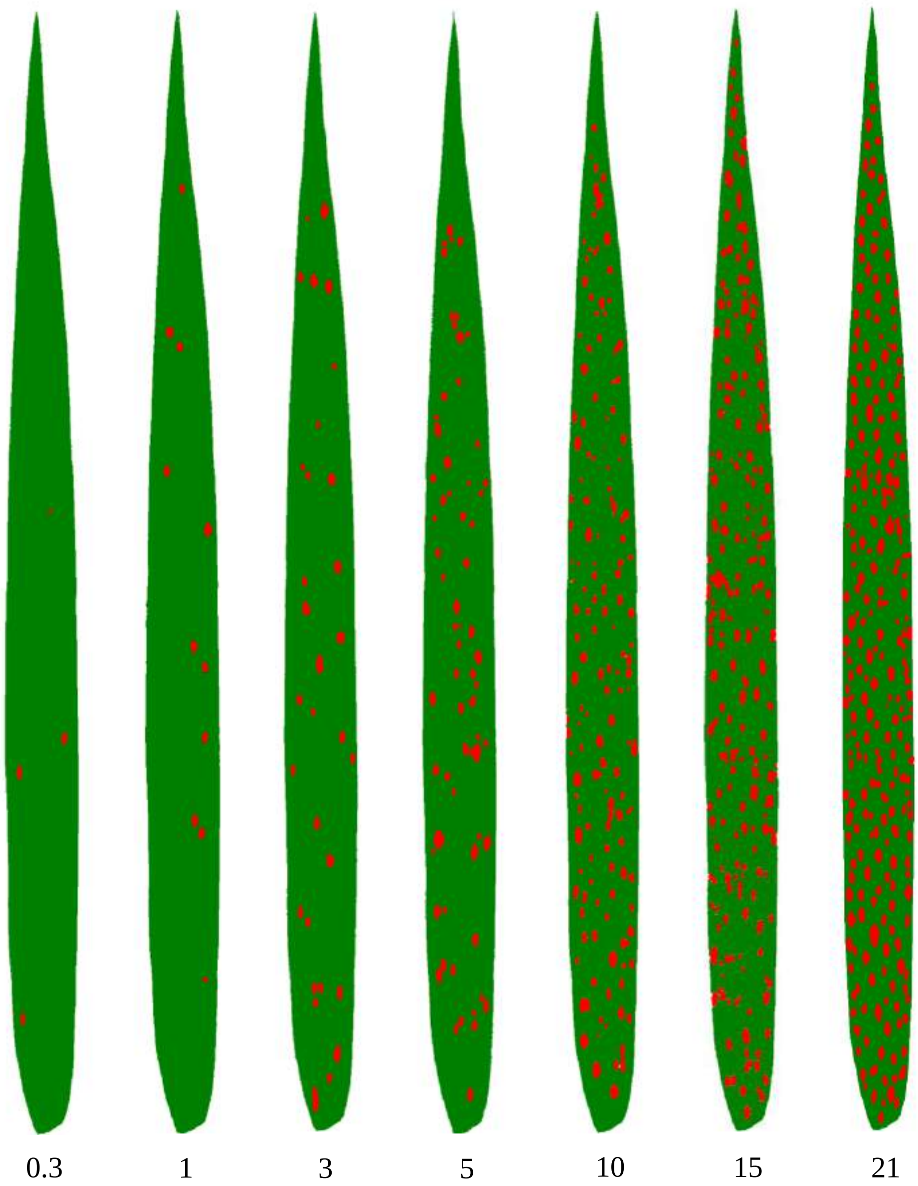

Fig. 2 Standard area diagram set for eyespot (*Drechslera gigantea*) severity on rice leaves. The number below each diagram represent percent leaf area with symptoms (circular spots with dark brown coloring).

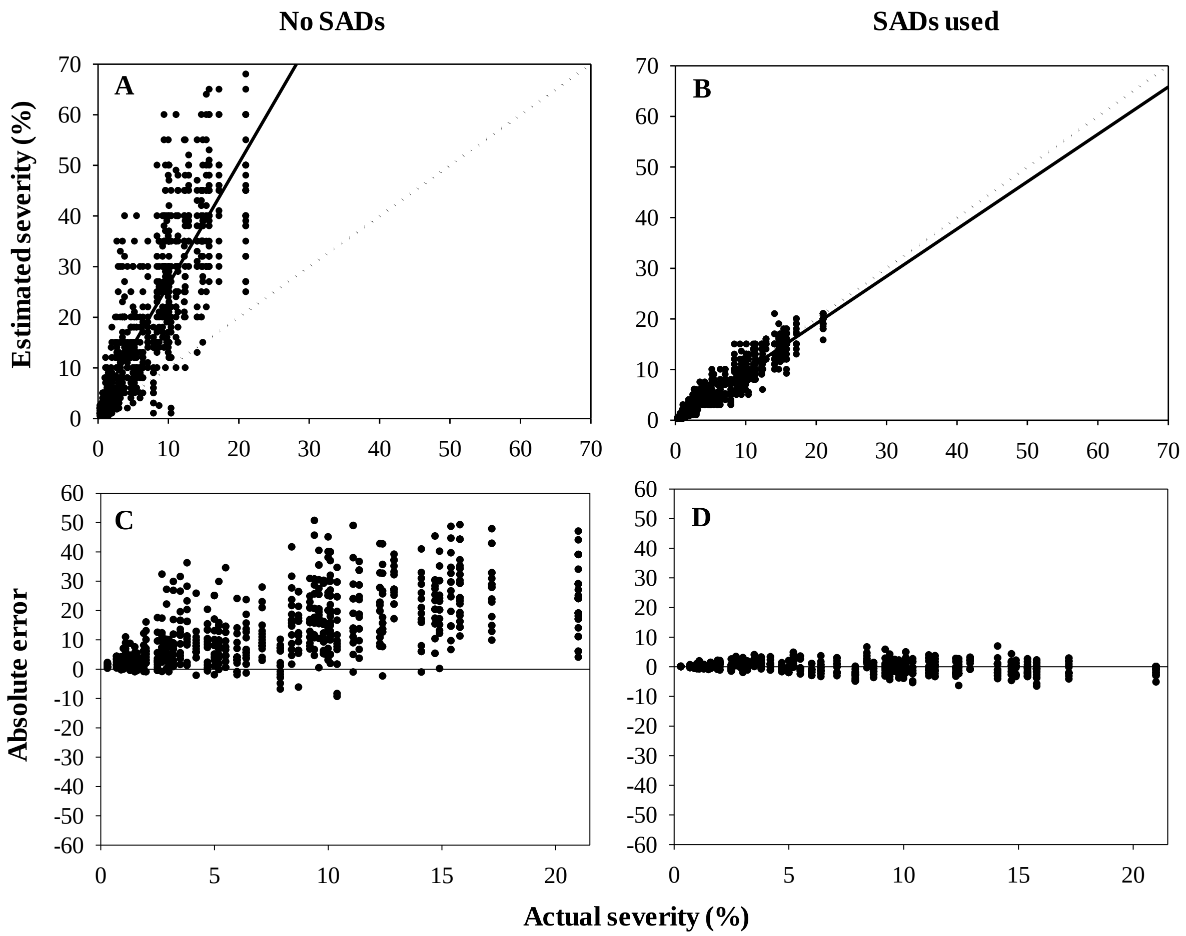

Fig. 3 Relationship between actual and estimated eyespot (*Drechslera gigantea*) severity on rice leaves without (a) and with (b) the use of a set of standard area diagrams (SADs) for 50 diseased leaves by 16 raters. The solid line represents the best-fitting line. The dashed line is the concordance line, which represents a perfect agreement between actual and estimated severity. Absolute error (estimated minus actual severity) of the estimates without SADs (c) and with the help of SADs (d) for the 50 diseased leaves.

4 Conclusões

Em conclusão, os resultados apresentados neste trabalho mostram que a aplicação de silicato de cálcio no solo resultou no acúmulo Si nas folhas de arroz o que incrementou a resistência à *Drechslera gigantea* na cultura do arroz.

Em condições de campo o efeito da fertilização silicatada foi menos expressivo.

A escala diagramática pode auxiliar em estimações mais acuradas, precisas e confiáveis, na quantificação da severidade da mancha ocular, portanto, poderá ser útil em estudos epidemiológicos, em pesquisas futuras.

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